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THE RESISTANCE OF THE GLANDERS BACILLUS TO CALCIUM HYPOCHLORITE

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A brief survey of available literature reveals a lack of definite information as to the resistance of the glanders bacillus to calcium hypochlorite (chlorid of lime), though there is a consensus of opinion among writers of textbooks on bacteriology that this organism is highly parasitic and quite sensitive to external physical and chemical agencies. For instance, a temperature below 22 C. is unfavorable for growth; and exposure to 75 C. for 1 hour, to 5% carbolic acid for ½ hour, or to 1:1,000 bichlorid of mercury for 15 minutes will destroy the organism.

The question of the longevity in water of the glanders bacillus has attracted attention because of its relation to the infection of horses through use of drinking troughs. In this regard it appears, according to Hiss and Zinsser, that the glanders bacillus may remain alive for 70 days in the water of horse troughs. It is therefore within the realm of possibility that an infection of a municipal water supply with this organism might be accomplished, whether by accident or intention. In view of such a possibility, however remote, it seemed desirable to determine the resistance of the glanders bacillus to hypochlorite in the strengths customarily employed for the disinfection of water supplies of cities.

The plan followed was to expose a suspension of the organism to concentrations of available chlorin of 0.10, 0.25, 0.50, 0.75, 1.00, and 2.00 parts per million, respectively, and determine at successive intervals the numbers of the survivors. From these data, curves may be constructed showing the corresponding rates of decline in numbers.

The culture was grown on a glycerol agar slant for 18 hours at 37.5 C.; and the growth was then washed off with 0.95% salt solution, the suspension being thoroughly shaken, and suitably diluted for the required tests. Of the hypochlorite, 0.3 gm. was dissolved in distilled water and filtered. To determine the amount of available chlorin it contained, this stock solution was titrated for each test with standard N/10 sodium thiosulfate. The calculated amount of stock hypochlorite solution was then accurately added to 100 c c of sterile tap water to give to it the required concentration of available chlorin. (Tap water was used to approximate natural conditions. One series

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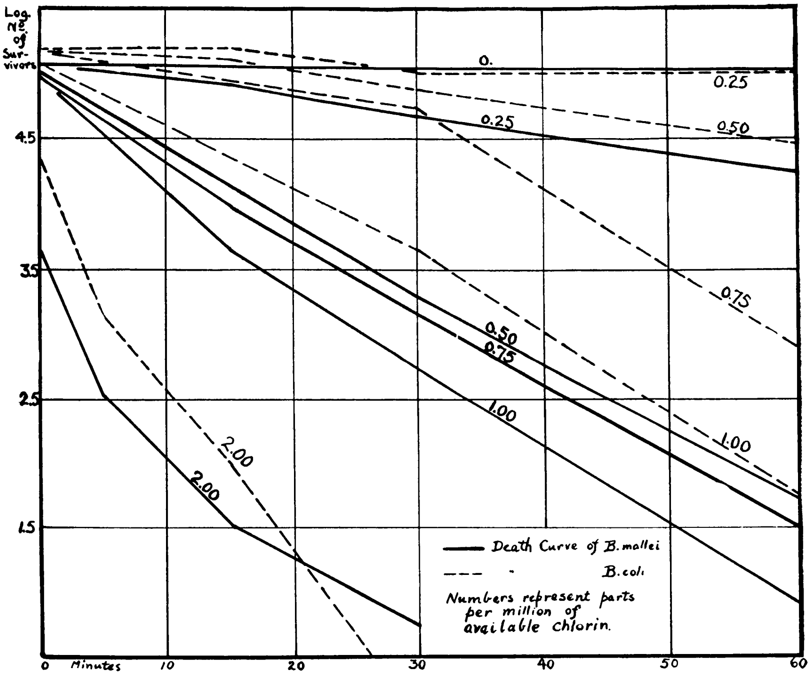
of tests in which distilled water was used, yielded practically the same results as those here reported.)

A small amount (0.1-1 c c) of the bacterial suspension was added to this last mentioned solution, the bottle quickly and thoroughly shaken, and 1 c c of the contents plated in suitable dilutions to determine the initial number of organisms present. The plating medium was 1% glycerol agar with a P_H of 6.6-7.0, and incubation of the plates was for 4 days at 37.5 C. The bottles were kept in diffuse light at room temperature (about 22 C.) which did not vary more than 2 or 3 degrees throughout the experiment; and at determined intervals up to 1 hour, samples were removed to determine the corresponding number of survivors. It was assumed that 1 hour was the maximum period of exposure that would normally occur in practice.



The rate of disinfection at the various concentrations of available chlorin.

Briefly, then, the procedure in these experiments was to (1) make the bacterial suspension; (2) titrate the stock hypochlorite solution; (3) prepare solution with known concentration of available chlorin; (4) add a little of the bacterial suspension to this solution of known chlorin content; (5) remove a 1 c c sample at suitable intervals and plate; (6) count the colonies on the plates after 4 days' incubation at 37.5 C.

It became evident early in these experiments that, to judge the resistance of the glanders bacillus properly, it was necessary to use another organism for comparison. Bacillus coli was chosen because of its known sensitiveness to calcium hypochlorite; and parallel tests were conducted on both B. mallei and B. coli.

Under the conditions of our experiments, there was only a slight or no diminution in numbers when the organisms were exposed to a concentration of $\frac{1}{10}$ part per million of available chlorin. This is probably accounted for by removal of the available chlorin by the relatively large amount of organic matter necessarily transferred in the bacterial suspension.

These results are not, therefore, directly comparable with those obtained in the disinfection of water supplies where (in the absence of much organic matter) small concentrations of chlorin are often effective. It is the comparative resistance of the two organisms which is significant. It will also be noted that concentrations of 0.5 or more parts per million of available chlorin are greater than normally used in the disinfection of water supplies. These were, however, included in this work in order to indicate the possible efficiency of hypochlorite in the disinfection of watering troughs.

TABLE 1

SURVIVORS PER C C OF B. MALLEI AND B. COLI AFTER EXPOSURE TO DIFFERENT CONCENTRATIONS OF 'AVAILABLE' CHLORIN FOR DIFFERENT PERIODS

Available Chlorin, Parts per Million	Time				
	0	5 Minutes	15 Minutes	30 Minutes	60 Minutes
B. Mallei					
0	111,000	.....	121,000	110,000	103,000
0.25	108,000	.....	84,000	47,000	16,500
0.50	101,000	.....	12,100	1,980	50
0.75	93,000	.....	11,500	1,640	29
1.00	95,000	.....	4,700	770	8
2.00	4,100	340	33	11	—
B. Coli					
0	138,000	.....	....	97,400	
0.25	142,000	.....	155,000	104,000	101,000
0.50	153,000	.....	127,000	74,000	29,000
0.75	139,000	.....	87,000	53,000	800
1.00	114,000	.....	....	4,600	55
2.00	21,000	1,400	100	0	—

Table 1 shows the results of a typical experiment in which the initial numbers of organisms were comparatively low (about 150,000 per c c). Another series of experiments not reported here in which large initial numbers (15 million per c c) of organisms were employed, gave substantially the same results, except the larger amount of organic matter caused a somewhat slower disinfection rate.

The accompanying chart gives a clear picture of the rate of disinfection at the various concentrations of available chlorin. The abscissae represent time in minutes, and the ordinates the logarithms of the numbers of survivors. Thus the slope of any curve in this chart shows the actual rate of decrease — the steeper the curve downward, the greater the rate of decrease.

The actual sensitivity of B. mallei to hypochlorite in a water supply, as pointed out in the foregoing, cannot be accurately judged from the death curves of this organism considered alone. The conditions of our experiments were such as to diminish greatly the effect of low concentrations of available chlorin; and even at the higher concentrations, one might be led to conclude erroneously that the glanders bacillus was quite resistant. When, however, the corresponding curves for B. mallei and B. coli are compared, it is seen that, if anything, B. mallei is more sensitive to hypochlorite than B. coli. It is well known that B. coli in water supplies is quite sensitive to hypochlorite in very low concentration, over 90% reduction being obtained in very short time. Therefore, we may conclude that under normal conditions of water-works practice in our larger cities, where the drinking water is continually chlorinated, B. mallei will have little chance to survive longer than B. coli.

It will also be noticed in the results here reported that practical sterility is gained in about 30 minutes with an exposure to 2 parts per million of available chlorin. This, however, applies only to a condition in which there is a rather light seeding of organisms and absence of considerable organic matter. Our evidence indicates, as would be expected, a diminution of disinfecting power of hypochlorite toward B. mallei in the presence of organic matter. This should be remembered when considering the question of the disinfection of horse troughs. In spite of this drawback, we have found that over-night exposure of B. mallei to 2 parts per million of available chlorin, even in the presence of considerable organic matter such as leaves and stems, has killed it, or at least effectively removed its power to grow when transferred to a favorable medium. One might reasonably conclude from this that sterilization of horse troughs could be easily effected with hypochlorite in relatively small amounts. Hypochlorite could be employed as a prophylactic measure in the water of horse troughs where glanders infection is prevalent.

SUMMARY

Studies were made on the specific resistance of the glanders bacillus toward calcium hypochlorite in concentrations employed for the disinfection of water supplies.

When compared to B. coli, B. mallei is, if anything, more sensitive toward hypochlorite.

Hypochlorite may be effectively used in the disinfection of horse troughs as a harmless prophylactic measure in glanders infected regions.